

Table S1. Cytokine stimulation in N protein treated monocyte-derived macrophages.

Cytokines	Stimulation	
	GM-CSF	M-CSF
IL-1 β	→	↑
IL-1 α	→	→
IL-2	↑	→
IL-4	↑	→
IL-5	↑	ND
IL-6	↑↑	↑
IL-7	→	→
IL-8	↑	↑↑
IL-9	→	↑
IL-10	→	→
IL-12(p70)	ND	ND
IL-13	→	→
IL-15	ND	ND
IL-17A	↑	→
Eotaxin	→	→
FGF basic	→	→
G-CSF	→	→
GM-CSF	added	→
IFN- γ	→	→
IP-10	↑	→
MCP-1(MCAF)	→	→
MIP-1 α	↑	→
PDGF-bb	→	→
MIP-1 β	↑	↑
RANTES	↑	↑
TNF- α	↑	↑
VEGF	ND	ND
HGF	→	(↓)
IL-18	ND	ND
TRAIL	↑	→
IL-2R α	ND	ND
M-CSF	→	added
GRO- α	↑	↑↑
MCP-3	→	→
MIG	↑	→
APRIL/TNFSF13	→	→
BAFF/TNFSF13B	↑	→
sCD30/TNFRSF8	ND	ND
sCD163	→	→
Chitinase 3-like 1	→	→
gp130/sIL-6rb	ND	ND
IFN- α 2	ND	ND
IFN- β	ND	ND
IL-6R α	→	→
IL-10	ND	↑
IL-11	→	→
IL-12(p40)	ND	ND
IL-19	ND	ND
IL-20	→	→
IL-22	↑	ND
IL-26	→	→
IL-27(p28)	ND	ND
IL-28A/IFN- α 2	→	ND
IL-29/IFN- λ 1	↑	ND
IL-32	ND	ND
IL-34	→	→
IL-35	ND	ND
LIGHT/TNFSF14	↑	ND
MMP-1	↑	ND
MMP-2	ND	ND
MMP-3	ND	ND
Osteocalcin	ND	ND
Osteopontin(OPN)	→	→
Pentraxin-3	↑	↑
sTNF-R1	→	→
sTNF-R2	→	→
TSLP	↑	↑↑
TWEAK/TNFSF12	→	→

↑ 1 : >2-fold increase at 156 and 625 ng/mL

↑ 2 : >2-fold increase at 625ng/mL

→ : <2-fold increase

ND: not detected

GM-CSF: granulocyte macrophage colony stimulating factor

M-CSF:macrophage colony stimulating factor

IL:interleukin

IFN: interferon

FGF:fibroblast growth factor

IP:interferon inducible protein

MCP: monocyte chemoattractant protein

MIP:macrophage inflammatory protein

PDGF:platelet-derived growth factor

RANTES:regulated on activation, normal T cell expressed and secreted

TNF: tumor necrosis factor

VEGF:vascular endothelial growth factor

HGF:hepatocyte growth factor

TRAIL:TNF-related apoptosis inducing ligand

GRO:growth-regulated oncogene product

MIG:monokine induced by INF γ

APRIL:A proliferation-inducing ligand

TNFSF:tumor necrosis factor ligand superfamily

BAFF:B-cell activating factor

LIGHT:lymphotoxin-related inducible ligand that competes for glycoprotein D binding to herpesvirus entry mediator on T cells

MMP:matrix metalloproteinase

TSLP:thymic stromal lymphopoietin

TWEAK:tumor necrosis factor-like weak inducer of apoptosis

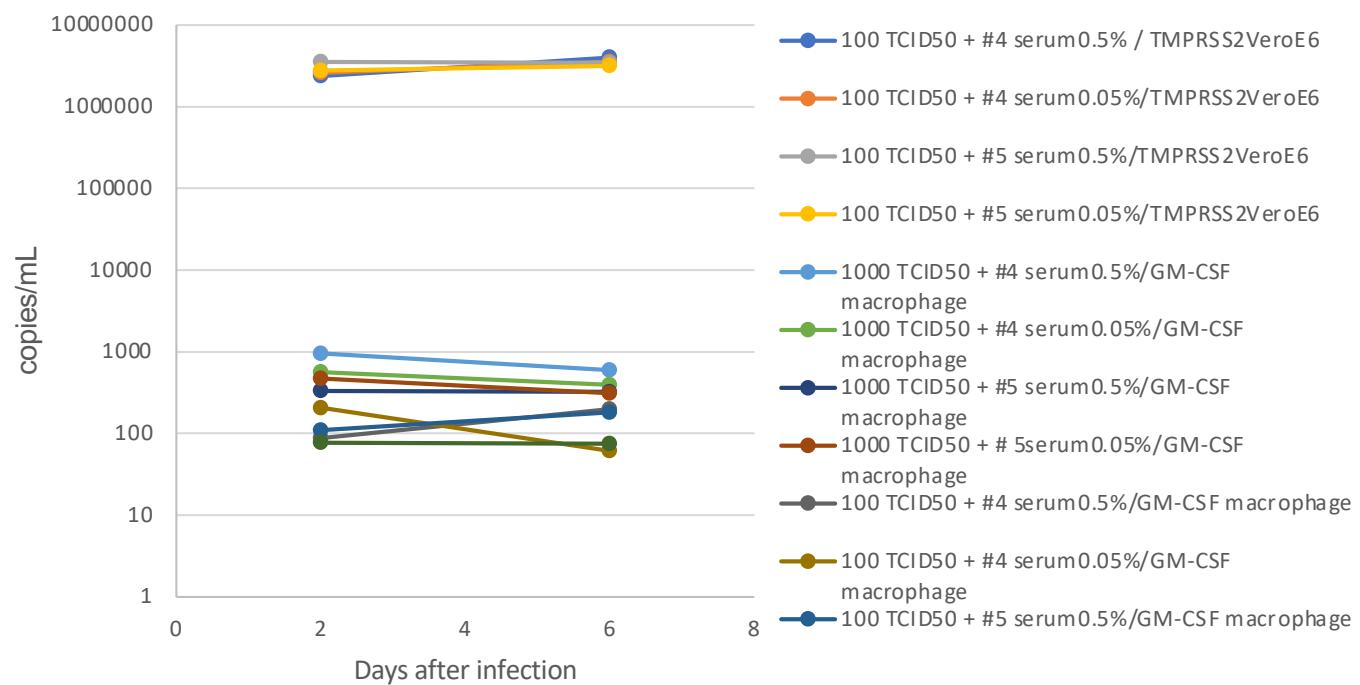
Table S2. List of monoclonal antibodies

	Clone	Subclass	Manufacturer
N1	HM1054	IgG2b	EastCoastBio
N2	HM1057	IgG1	EastCoastBio
N4	3851	IgG2b	Virostat
N5	A72061	IgG1	BiosPacific
N6	A03092	IgG2a	BiosPacific
N7	A03090	IgG2a	BiosPacific
N8	A03091	IgG2a	BiosPacific
N9	HM1058	IgG1	EastCoastBio
N10	HM1063	IgG2a	EastCoastBio
N11	HM1065	IgG2a	EastCoastBio
N12	A72063	IgG1	BiosPacific
S2	S2-2-5-D6-3	IgG1	RIMD
C1	CV10	IgG2a	Certest
C2	CV15	IgG1	Certest
C3	B3449M	IgG1	MeridianLifescience
C4	B3451M	IgG1	MeridianLifescience

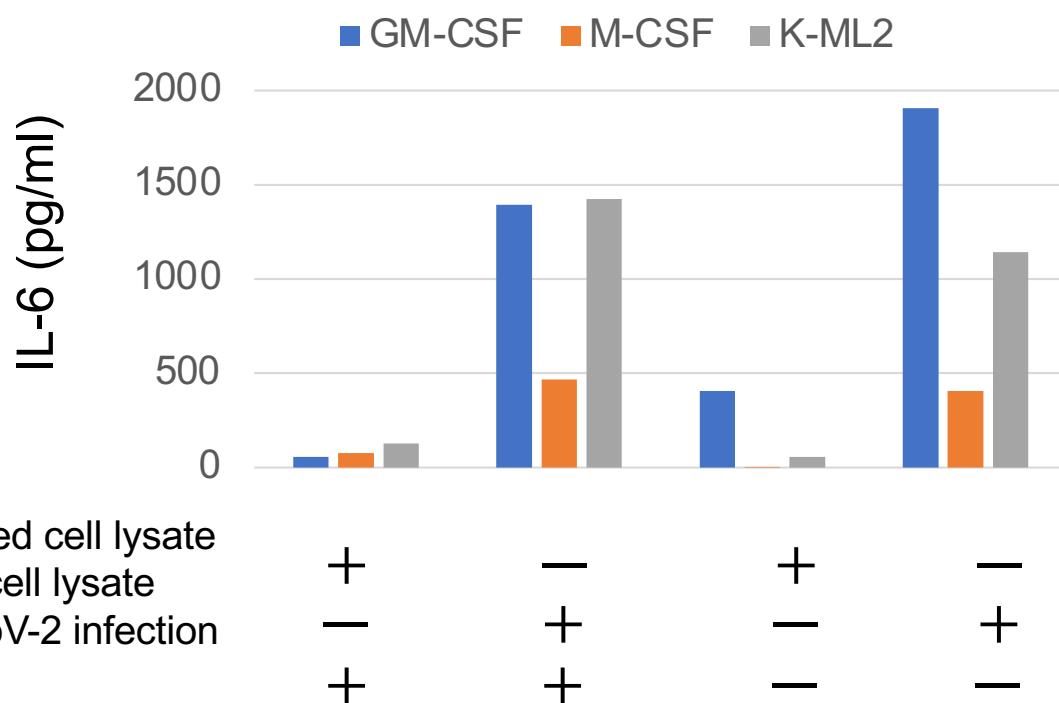
Table S3. List of plasmids

Catalog No.	Name
1	141395 pLVX-EF1alpha-eGFP-2xStrep-IRES-Puro
2	141391 pLVX-EF1alpha-SARS-CoV-2-N-2xStrep-IRES-Puro
3	141375 pLVX-EF1alpha-SARS-CoV-2-nsp9-2xStrep-IRES-Puro
4	141387 pLVX-EF1alpha-SARS-CoV-2-orf6-2xStrep-IRES-Puro
5	141389 pLVX-EF1alpha-2xStrep-SARS-CoV-2-orf7b-IRES-Puro
6	141393 pLVX-EF1alpha-2xStrep-SARS-CoV-2-orf9c-IRES-Puro
7	141388 pLVX-EF1alpha-SARS-CoV-2-orf7a-2xStrep-IRES-Puro
8	141394 pLVX-EF1alpha-SARS-CoV-2-orf10-2xStrep-IRES-Puro
9	141376 pLVX-EF1alpha-SARS-CoV-2-nsp10-2xStrep-IRES-Puro
10	141369 pLVX-EF1alpha-SARS-CoV-2-nsp4-2xStrep-IRES-Puro
11	141373 pLVX-EF1alpha-SARS-CoV-2-nsp7-2xStrep-IRES-Puro
12	141379 pLVX-EF1alpha-SARS-CoV-2-nsp13-2xStrep-IRES-Puro
13	141367 pLVX-EF1alpha-SARS-CoV-2-nsp1-2xStrep-IRES-Puro
14	141383 pLVX-EF1alpha-SARS-CoV-2-orf3a-2xStrep-IRES-Puro
15	141374 pLVX-EF1alpha-SARS-CoV-2-nsp8-2xStrep-IRES-Puro
16	141385 pLVX-EF1alpha-SARS-CoV-2-E-2xStrep-IRES-Puro
17	141381 pLVX-EF1alpha-SARS-CoV-2-nsp15-2xStrep-IRES-Puro
18	141390 pLVX-EF1alpha-SARS-CoV-2-orf8-2xStrep-IRES-Puro
19	141377 pLVX-EF1alpha-SARS-CoV-2-nsp11-2xStrep-IRES-Puro
20	141370 pLVX-EF1alpha-SARS-CoV-2-nsp5-2xStrep-IRES-Puro
21	141378 pLVX-EF1alpha-SARS-CoV-2-nsp12-2xStrep-IRES-Puro
22	141392 pLVX-EF1alpha-SARS-CoV-2-orf9b-2xStrep-IRES-Puro
23	141380 pLVX-EF1alpha-2xStrep-SARS-CoV-2-nsp14-IRES-Puro
24	141371 pLVX-EF1alpha-SARS-CoV-2-nsp5-C145A-2xStrep-IRES-Puro
25	141368 pLVX-EF1alpha-SARS-CoV-2-nsp2-2xStrep-IRES-Puro
26	141386 pLVX-EF1alpha-SARS-CoV-2-M-2xStrep-IRES-Puro

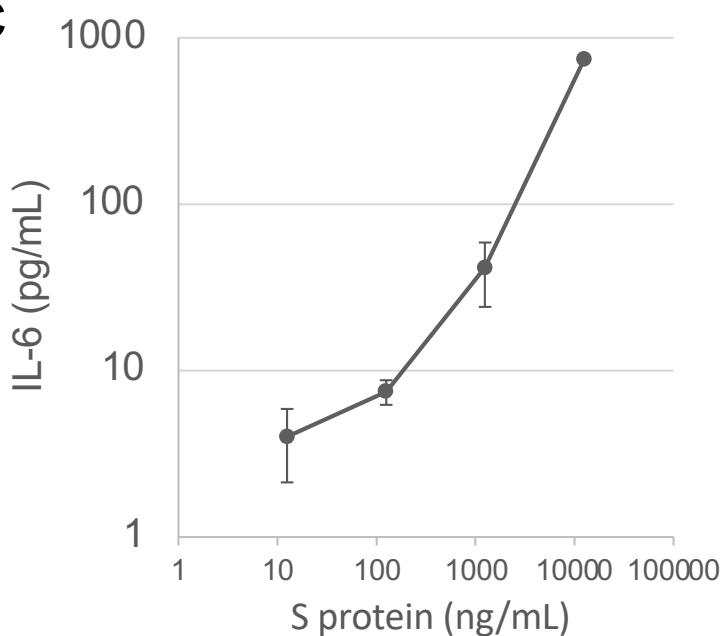
S1
A



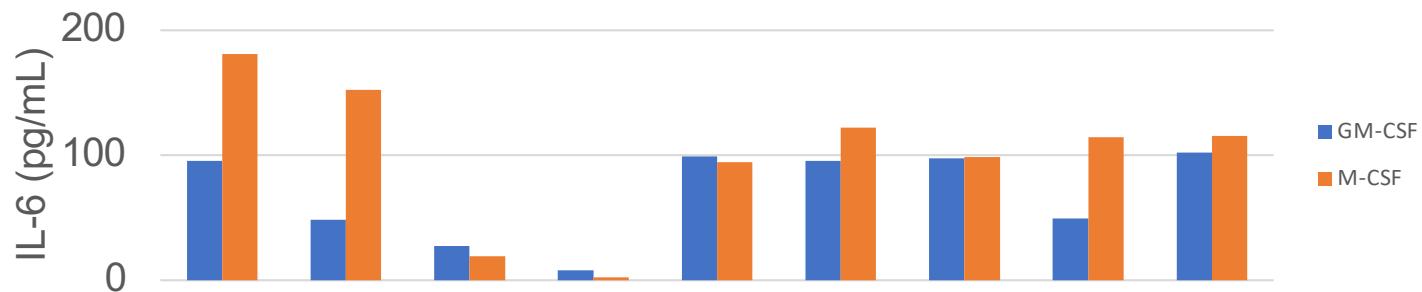
B



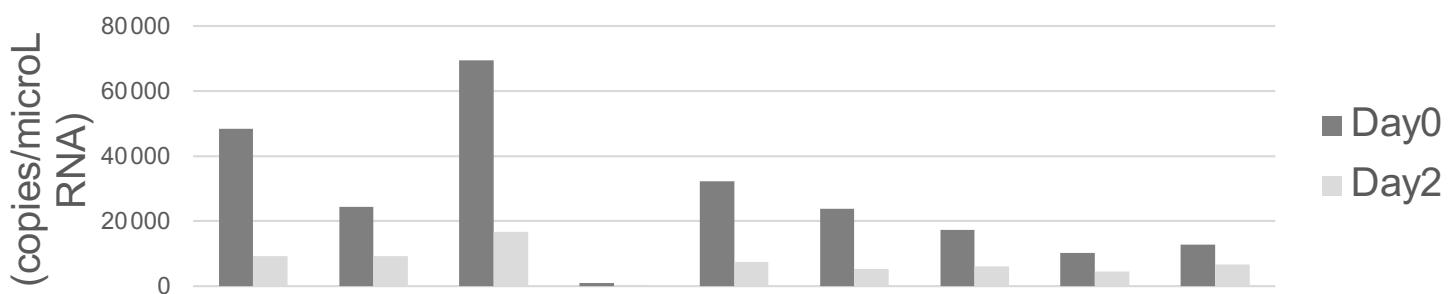
C



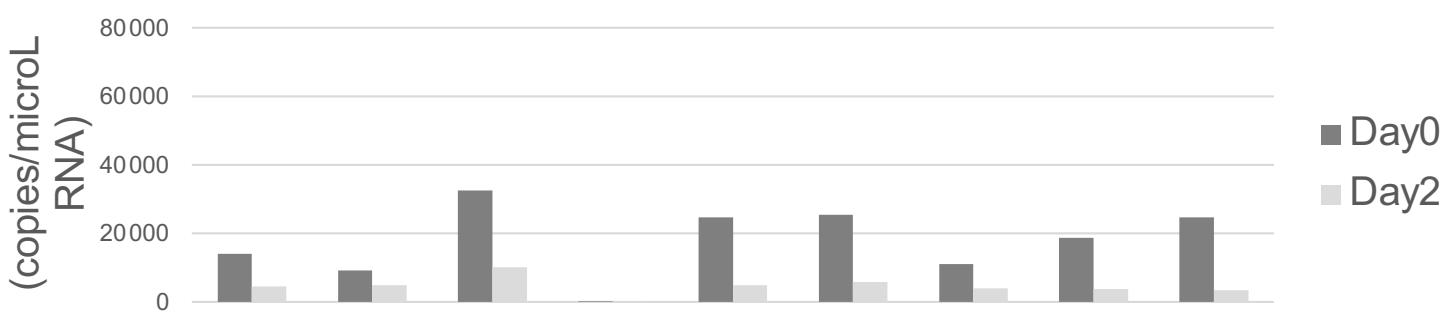
(serum+lysate) & SRAS-CoV-2



GM-CSF macrophage



M-CSF macrophage



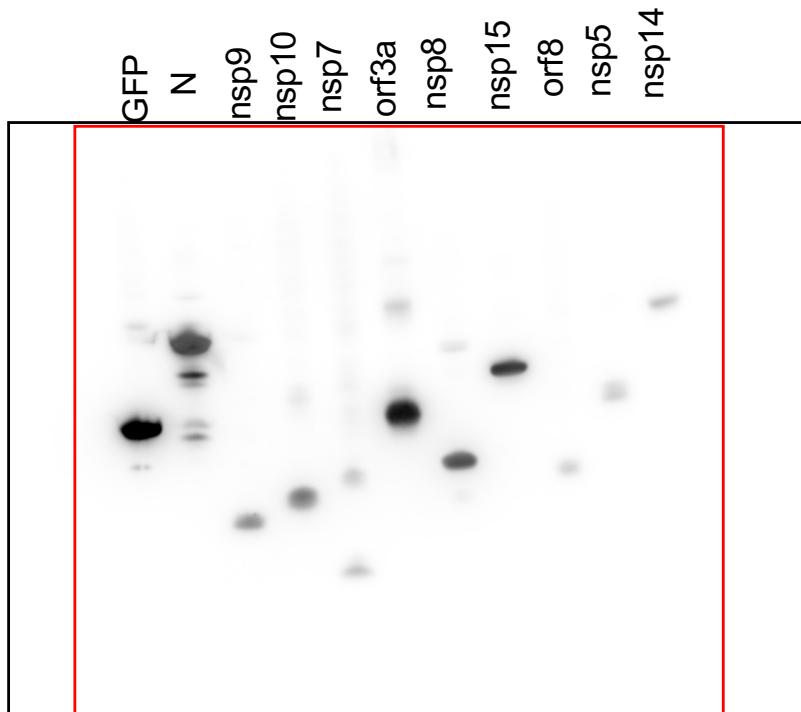
	(-)	(-)	(-)	(-)	HD 2	HD 1	HD 3	HD 4	HD 5
Serum	(-)	(-)	(-)	(-)					
lysate	(+)	A.1	B.1.1.24 8	(-)	(+)	(+)	(+)	(+)	(+)
over-infection	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)

(+) KNG19-020 strain

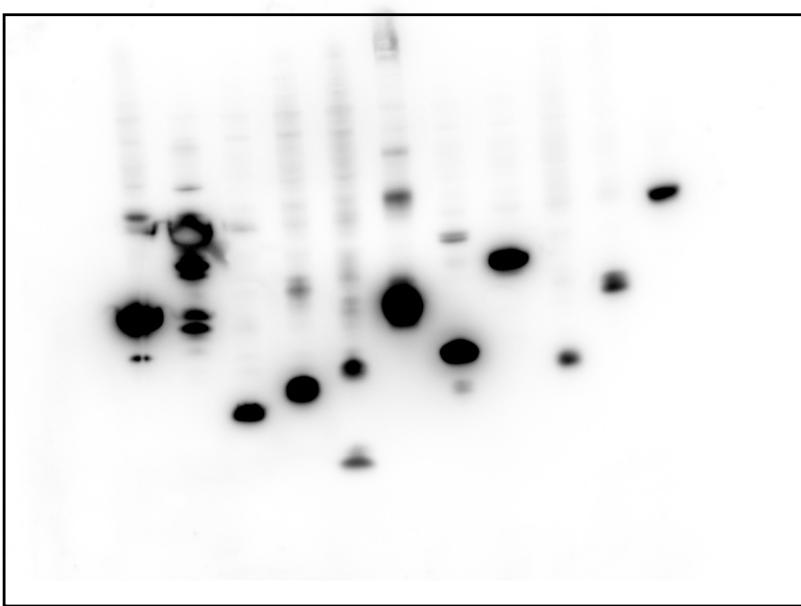
HD: Healthy donor

S3A

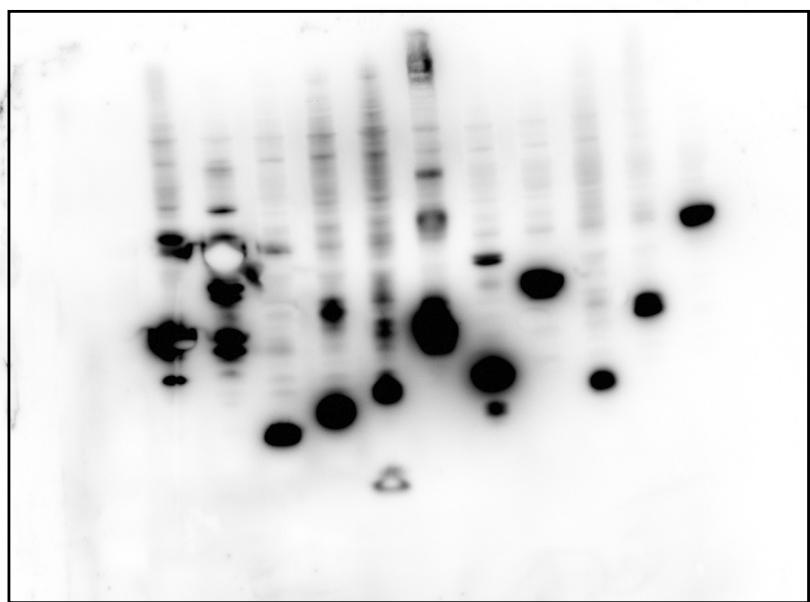
30sec



3min



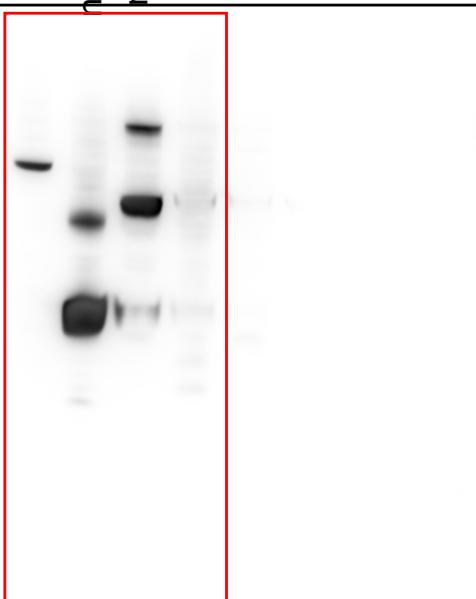
10min



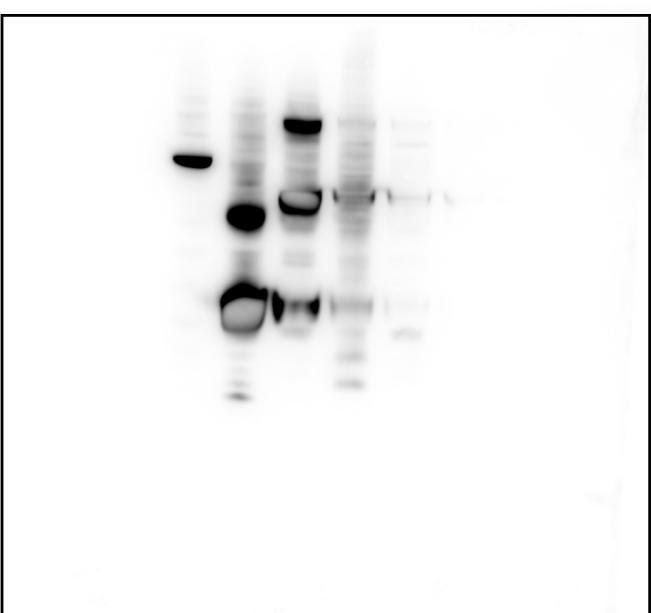
S3B

30sec

nsp12
nsp5C145A
nsp2
M



3min



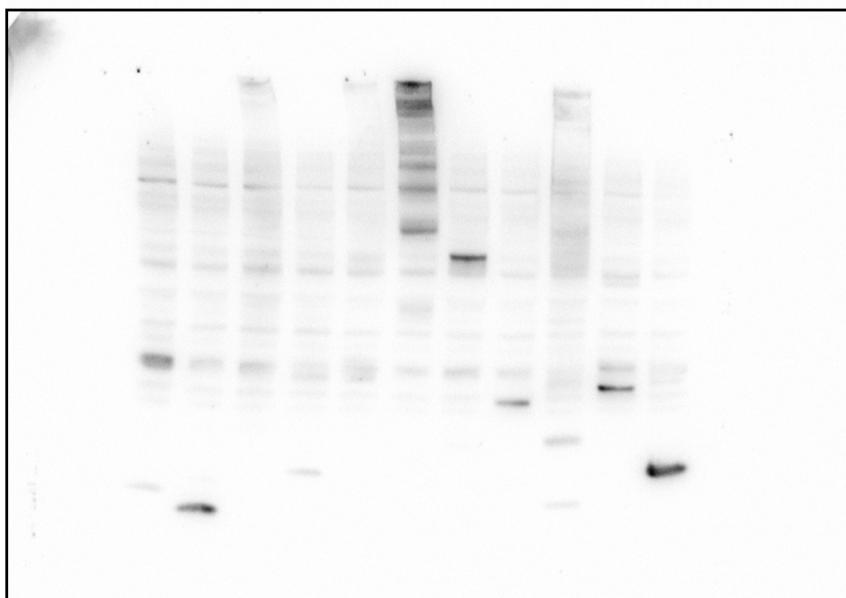
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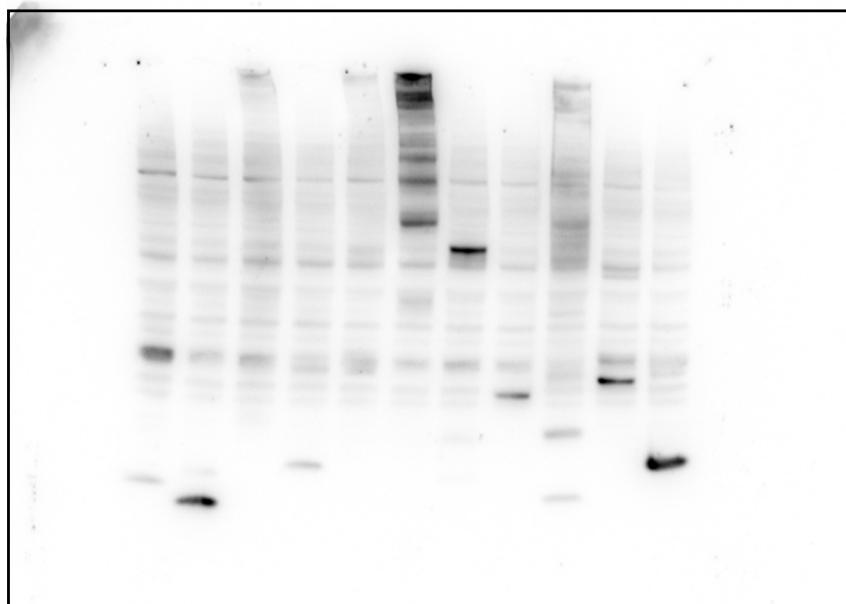
S3C

orf6 orf7b orf9c orf7a orf10 nsp4 nsp13 nsp1 E nsp11 orf9b

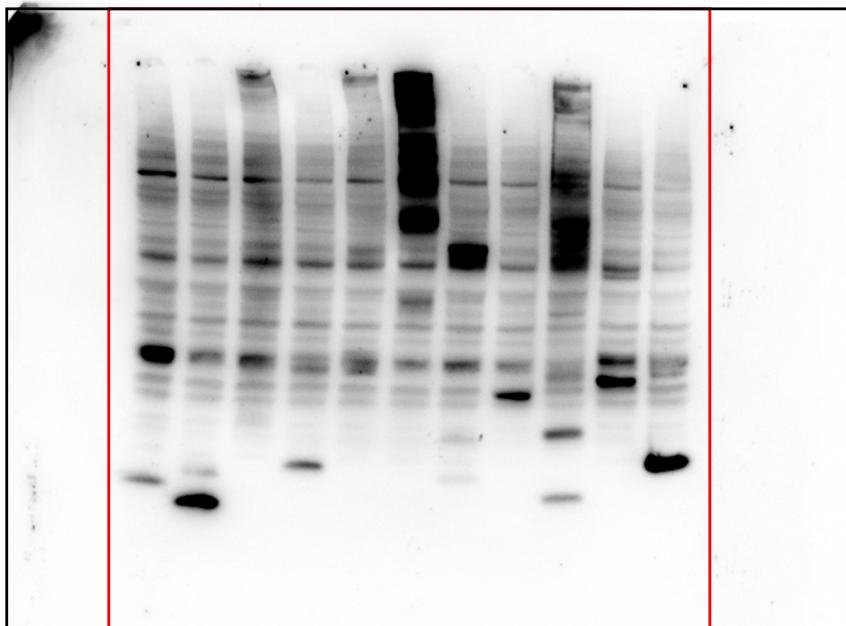
30sec



3min

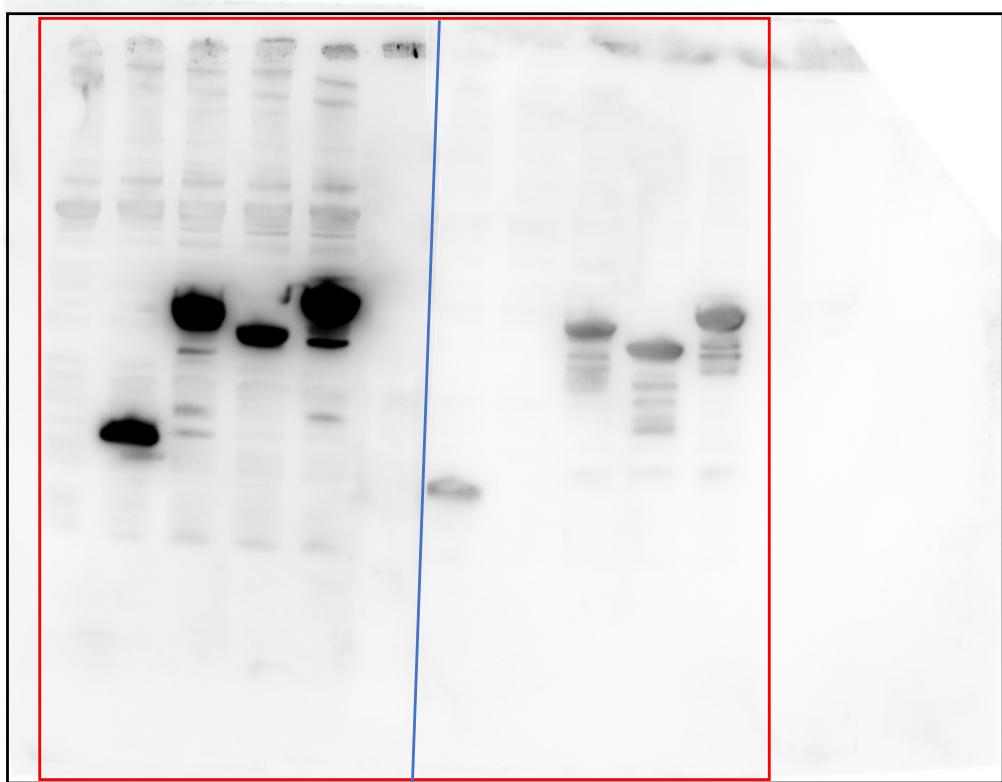


10min

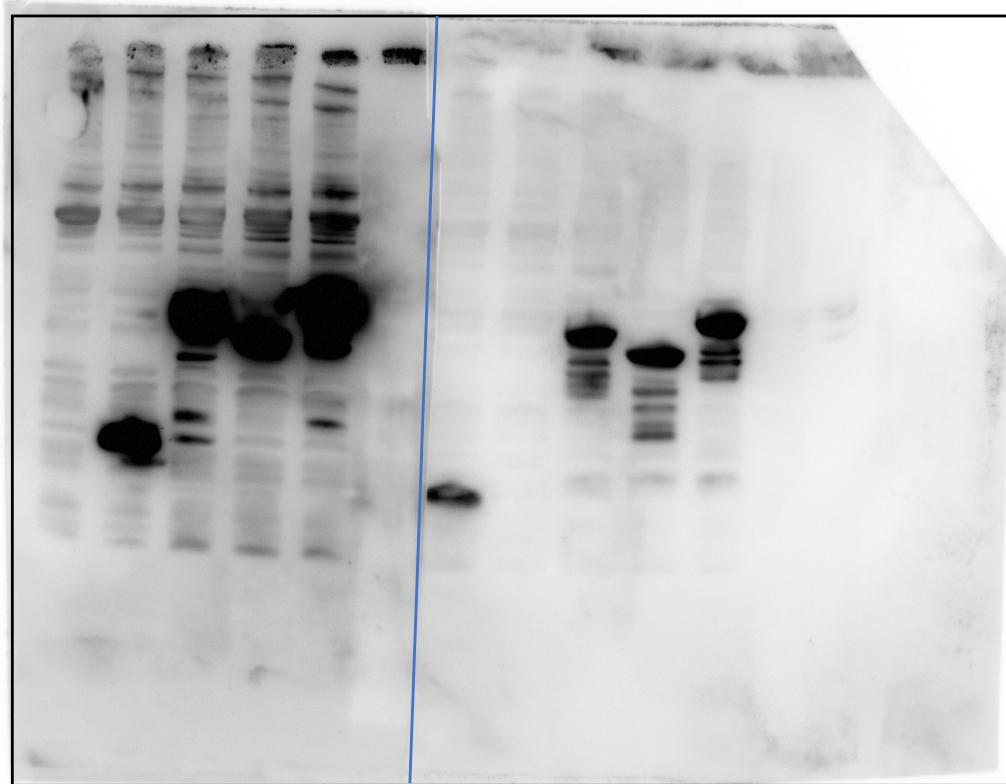


S3D
3min

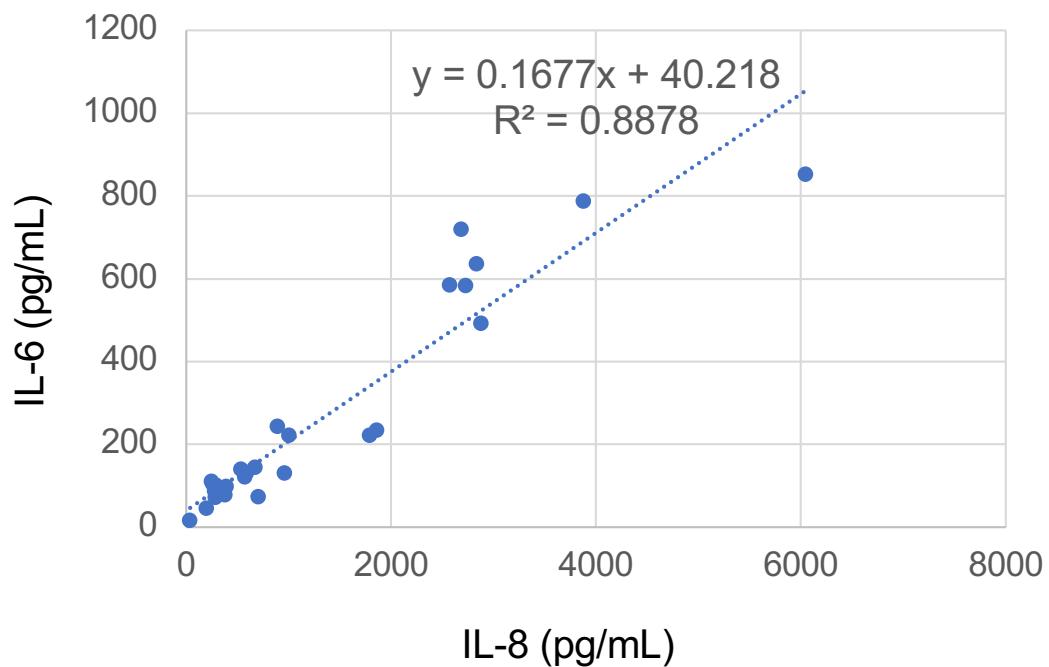
NTD CTD Full NTD-CTD N Marker NTD CTD Full NTD-CTD N



10min



A



B

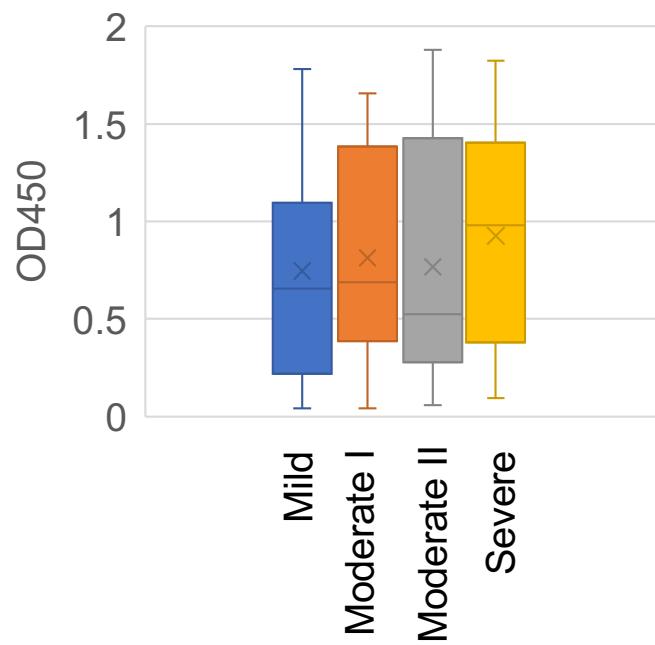


Figure S1

(A) TMPRSS2/VeroE6 cells and macrophages differentiated with GM-CSF were infected with 1000 50% tissue culture infectious dose (TCID₅₀) or 100 TCID₅₀ of SARS-CoV-2 (JPN-TY-Wk-521 strain) together with COVID-19 patient sera. Four hours later, cells were washed once. The viral RNA in the culture supernatants at days 2 and 6 was measured by RT-PCR. (B) iPS-derived myeloid cells K-ML2 (gray) and MDM (blue and red) were inoculated with the SARS-CoV-2 KNG-19-020 strain with SARS-CoV-2-infected or uninfected cell lysate. Four hours later, the MDM were washed once. Two days after infection, the IL-6 levels in the culture supernatants were measured by ELISA. (C) K-ML2 cells were stimulated with S protein for 2 days, and the IL-6 levels in the culture supernatants were measured by ELISA. The means and standard deviation of triplicate samples are shown.

Figure S2

TMPRSS2/VeroE6 cells were infected with the SARS-CoV-2 KNG19-020 strain, clinical isolate hCoV-19/Japan/OIPH14/2020 (A.1. lineage) or hCoV-19/Japan/OIPH21/2020 (B.1.1.214 lineage). On day 1, the cells were harvested and lysed by freeze-thawing. Macrophages differentiated with GM-CSF or M-CSF were infected with the SARS-CoV-2 KNG19-020 strain together with cell lysate and 0.5% serum from healthy donors. The negativity for SARS-CoV-2 infection of these healthy donors was confirmed by immunostaining of SARS-CoV-2-infected TMPRSS2/VeroE6 cells with these sera. After 4 h of incubation, the cells were washed once. The IL-6 levels in the culture supernatants at day 2 were measured by ELISA. The viral RNA in the remaining culture supernatants was measured by RT-PCR.

Figure S3

The original images of western blot assay presented in Figure 2B (A, B, C) and Figure 2E (D). The blotted membranes were developed for 30 sec, 3 min, and 10 min until the edges were visible. The black boxes denote the outline of the edges of the blots. The regions used for Figure 2B and 2E were denoted by red boxes. Blue vertical dividing lines of two membranes are shown in panel D.

Figure S4

- (A) Elevated levels of IL-8 and IL-6 in the culture supernatant of K-ML2 cells treated with N protein (156 ng/mL) in the presence of 1% serum from 24 patients. The correlation between IL-8 and IL-6 levels is significant ($R=0.94$, $P<0.0001$).
- (B) The levels of anti-N antibody in the patient sera were measured by an in-house ELISA. The center lines in the boxes and the boxes indicate the medians and 25/75 percentiles, respectively.